Citation:

Albert CM, Cook NR, Gaziano JM, Zaharris E, MacFadyen J, Danielson E, Buring JE, Manson JE. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: A randomized trial. *JAMA.* 2008; 299: 2,027-2,036.

PubMed ID: 18460663

Study Design:

Randomized controlled trial (individual randomized)

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To determine whether a combination of folic acid (2.5mg per day), vitamin B_6 (50mg per day) and vitamin B_{12} (1.0mg per day) lowered risk of cardiovascular disease (CVD) among high-risk women with and without CVD over 7.3 years of follow-up.

Inclusion Criteria:

- Participants from the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS):
  - Aged 40 years or older
  - Post-menopausal or had no intention of becoming pregnant
  - Had a reported history of CVD or had at least three cardiac risk factors. CVD was defined as a reported history of myocardial infarction (MI), stroke, coronary or peripheral revascularization, angina pectoris or transient ischemic attack. Qualifying cardiac risk factors were diagnosed hypertension, high cholesterol, diabetes mellitus, parental history of premature MI (i.e., younger than 60 years), obesity [body mass index of 30kg/m^2 or more (calculated as weight in kilograms divided by height in meters squared)] and current cigarette use
- Women willing to take individual supplements of folic acid, vitamin B_6, and vitamin B_{12} at levels beyond the US recommended daily allowance (RDA) of 400mcg of folic acid, 2.0mg of vitamin B_6, or 6mcg of vitamin B_{12} during the trial and had multivitamin use at or below these RDA levels was allowed
- Women who provided a blood sample in 1996 were eligible for the blood sub-study.

Exclusion Criteria:

- History of cancer (excluding non-melanoma skin cancer) within the past 10 years
- Any serious non-CVD illness

- Currently using warfarin or other anticoagulants.

Description of Study Protocol:

Recruitment

Women in the Women's Antioxidant and Folic Acid Cardiovascular study.

Design

Randomized controlled trial (individuals randomized).

Dietary Intake/Dietary Assessment Methodology

Semi-quantitative food frequency questionnaire (FFQ).

Blinding Used

All study investigators, personnel and participants were unaware of the participants' treatment assignments. Physicians who adjudicated all primary and secondary cardiovascular outcome events were blinded to randomized treatment assignment.

Intervention

Daily placebo or a combination pill containing 2.5mg of folic acid, 50mg of vitamin B_6 and 1.0mg of vitamin B_{12} (active treatment).

Statistical Analysis

- SAS version 9 using two-sided tests with a significance level of 0.05
- Baseline characteristics: T-tests, X^2 tests for proportions and tests for trend for ordinal categories
- Primary and secondary outcomes:
    - Intent-to-treat basis
    - Person-time was calculated until the first confirmed end point specified by the analysis or to the end of the trial if no endpoint occurred
    - Kaplan-Meier curves: Estimate cumulative incidence over time by randomized

treatment group and the log-rank test was used to compare survival curves
    - Cox proportional hazards models were used to calculate RRs expressed as hazard ratios and 95% CI, after adjustment for age and other randomized treatment assignments (vitamin E, vitamin C and beta carotene)
    - Proportionality assumption was tested using an interaction term for treatment with log time, and was met for each of the primary and secondary analyses
    - Effect of non-adherence, a post-hoc sensitivity analysis censored participants when they stopped taking at least two-thirds of their study medications, reported taking outside supplements or were missing adherence
- Pre-specified subgroup analyses:
    - Stratified Cox proportional hazards models: Antioxidant treatment assignment(s), presence or absence of prior CVD, dietary folic acid intake, smoking, diabetes, aspirin, hormone therapy and multivitamin use were performed
    - Analyses used baseline exposure assessments and were restricted to participants with non-missing subgroup data at baseline
- Exploratory subgroup analyses:
    - Conducted to evaluate the consistency of the results
    - Tests for effect modification by subgroup used interaction terms between subgroup indicators and randomized assignment, with a test for trend for ordinal subgroup categories
    - Raw distributions and median values of plasma homocysteine and folate levels in the blood substudy were compared using the non-parametric Wilcoxon rank sum test
    - Homocysteine, geometric means were compared after natural logarithmic transformation to compare differences between treatment groups.

Data Collection Summary:

Timing of Measurements

- 7.5 years of follow-up
- Randomization began in April 1998 and ended in July 31, 2005.

Dependent Variables

- Primary: Combined endpoint of cardiovascular morbidity and mortality, including incident MI, stroke, coronary revascularization procedures (coronary artery bypass grafting or percutaneous coronary intervention) and cardiovascular mortality
- Secondary: Individual components of total MI, total stroke and total coronary heart disease events (MI, coronary revascularization, and death from coronary heart disease)
- Dietary intake.

Independent Variables

Placebo vs. active treatment.

Control Variables

Cox proportional hazards models were adjusted for age and other randomized treatment assignments (vitamin E, vitamin C and beta carotene).

Description of Actual Data Sample:

- *Initial N:* 5,442 (100% female)
- *Attrition:* 5,442 (mortality and morbidity information was complete for 98.9% and 98.0%, respectively, of person-years follow-up for the group randomized to receive the treatment (N=2,721) and placebo (N=2,721)
- *Age:* Range from 45 to 65 years or older; mean (SD) was 62.8 (8.8) years
- *Anthropometrics:*
  - No significant difference between treatment and placebo groups in baseline characteristics for age, prior cardiovascular disease, hypertension, elevated cholesterol, BMI, parental history of MI, alcohol intake, diabetes, current smoking status, current mediation use (aspirin, hormone therapy, lipid-lowering drugs, beta blockers, angiotensin converting enzyme inhibitors, multivitamins), median dietary intake (folic acid, vitamin B_{12}, vitamin B_6)
  - 64.2 % of women had history of CVD
- *Location:* United States.

Summary of Results:

- The mediam daily dietary intake of folic acid including supplements was 432mcg
- Adherence: Self-reported and defined as taking at least two-thirds of the study pills. Average adherence was 83% for active and placebo group and no significant difference between groups
- Use of open-label folic acid supplements, vitamin B_6, or vitamin B_{12} supplements containing more than the RDA for at least four days per month were:
  - Active group, 2% to 11%
  - Placebo group, 2% to 13%
- There were no serious adverse events reported that were conclusively related to study interventions.

Primary Analysis

- 796 participants (14.6%) experienced a confirmed CVD event (139 MIs, 148 strokes, 508 coronary revascularization procedures and 190 cardiovascular deaths)
- No difference in the cumulative incidence of the primary combined endpoint in the active vs. placebo treatment groups at any time during study follow-up
- 406 women (14.9%) in the active group and 390 (14.3%) in the placebo group experienced at least one cardiovascular event included in the primary endpoint (226.9 of 10,000 person-years vs. 219.2 of 10,000 person-years). Overall RR of 1.03 (95% CI, 0.90 to 1.19; P=0.65) after controlling for age and antioxidant treatment assignment
- No evidence for a treatment effect in sensitivity analysis censoring at non-adherence (RR, 1.05; 95% CI, 0.90 to 1.23; P=0.53) or if coronary revascularization procedures were excluded from the primary endpoint (RR, 0.96; 95% CI, 0.80 to 1.17; P=0.72).

Secondary and Other Outcomes

- Total coronary heart disease events: Active group had 283 participants with CAD events (156.5 of 10,000 person-years) and placebo group had 280 participants with CAD events (155.8 of 10,000 person-years) (RR, 1.00; 95% CI, 0.85 to 1.18; P=0.96)
- Separate analysis: No significant differences between groups for each of the components of the primary outcome, including MI (34.5 of 10,000 person-years vs. 39.5 of 10,000 person-years; RR, 0.87; 95% CI, 0.63 to 1.22; P=0.42), stroke (41.9 of 10 000 person-years vs. 36.8 of 10,000 person-years; RR, 1.14; 95% CI, 0.82 to 1.57; P=0.44) and CVD mortality (50.3 of 10,000 person-years vs. 49.6 of 10,000 person-years; RR, 1.01; 95% CI, 0.76 to 1.35; P=0.93)
- No difference between groups for risk of death from any cause (RR, 0.97; 95% CI, 0.81-1.15; P =0.73).

Relative Risks Of Clinical Outcomes According To Treatment Assignment with Folic Acid, Vitamin B_6 and Vitamin B_{12} vs. Placebo

Outcome	Active N=2,721	Placebo N=2,721	Relative Risk* (95% CI)	P-value
	No. of Patients (%)			
Combined Major Cardiovascular Disease†	406 (14.9)	390 (14.3)	1.03 (0.90–1.19)	0.65
Myocardial Infarction	65 (2.4)	74 (2.7)	0.87 (0.63–1.22)	0.42
Stroke	79 (2.9)	69 (2.5)	1.14 (0.82–1.57)	0.44
Ischemic‡	69 (2.5)	62 (2.3)	1.10 (0.78–1.56)	0.57
Hemorrhagic‡	10 (0.4)	6 (0.2)	1.65 (0.60–4.53)	0.33
Coronary Revascularization§	253 (9.3)	255 (9.4)	0.99 (0.83–1.17)	0.87
Coronary Artery Bypass Grafting‖	87 (3.2)	98 (3.6)	0.88 (0.66–1.17)	0.38
Percutaneous Coronary Intervention‖	192 (7.1)	177 (6.5)	1.08 (0.88–1.33)	0.46
Cardiovascular death	96 (3.5)	94 (3.5)	1.01 (0.76–1.35)	0.93
Myocardial infarction, stroke, and cardiovascular death	205 (7.5)	211 (7.8)	0.96 (0.80–1.17)	0.72
Total coronary heart disease#|	283 (10.4)	280 (10.3)	1.00 (0.85–1.18)	0.96
Total mortality	250 (9.2)	256 (9.4)	0.97(0.81–1.15)	0.73

*Estimated from Cox proportional hazards models that adjusted for age and randomized treatment assignment to vitamin E, vitamin C and beta-carotene.
† The primary outcome is defined as a composite endpoint comprising the first of any of these events: Non-fatal myocardial infarction, stroke, coronary revascularization procedures (coronary artery bypass grafting or percutaneous coronary intervention) and cardiovascular mortality.
‡ Stroke type was unknown for one woman in the placebo group.

§Composite endpoint comprised of the first coronary artery bypass grafting or percutaneous coronary intervention.
‖Includes all incident coronary artery bypass grafting operations and percutaneous coronary intervention, respectively.
#Composite endpoint comprised of the first of any of these events: Non-fatal MI, coronary revascularization procedures (coronary artery bypass grafting or percutaneous coronary intervention) and coronary heart disease death.

Distribution of Plasma Levels of Folate and Homocysteine in 300 Participants in the Blood Sub-Study at Baseline Prior to Randomization and at the End of Treatment and Follow-up

	Baseline		Follow-up	
Characteristic	**Placebo (N=150)**	**Active (N=150)**	**Placebo (N=150)**	**Active (N=150)**
Folate (ng per ml)				
Less than 7	52 (34.7)	49 (32.7)	2 (1.33)	0 (0.00)
7 to 15	71 (47.3)	80 (53.3)	69 (46.0)	1 (0.67)
15 to 25	22 (14.7)	19 (12.7)	54 (36.0)	21 (14.0)
25 to 40	4 (2.67)	2 (1.33)	18 (12.0)	54 (36.0)
40 or more	1 (0.67)	0 (0.00)	7 (4.67)	74 (49.3)
Homocysteine (µmol per L)				
Less than 9	30 (20.0)	21 (14.0)	21 (14.0)	64 (42.7)
9 to 12	40 (26.7)	52 (34.7)	57 (38.0)	43 (28.7)
12 to 15	35 (23.3)	39 (26.0)	35 (23.3)	28 (18.7)
15 or more	45 (30.0)	38 (25.3)	37(24.7)	15 (10.0)

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Other Findings

- Subgroup analyses:
  - No significant treatment between groups for the primary outcome in any of the pre-specified or exploratory subgroups evaluated
  - There was a similar lack of benefit among participants without prior CVD as compared with those with prior CVD (P=0.93 for interaction), although the trial was not powered to detect a specific benefit in this subgroup
  - No evidence that either dietary folate intake or multivitamin use modified the treatment effect, although power was limited for these subgroups as well
  - Test for interaction was significant for treatment with angiotensin-converting enzyme inhibitors (P=0.03); however, this was not a pre-specified subgroup analysis
- Effect of supplementation vs. fortification on folate and homocysteine levels: Geometric mean homocysteine level was decreased by 18.5% (95% CI, 12.5% to 24.1%; P<0.001) in

the active group over that observed in the placebo group for a difference of 2.27μmol per L (95% CI, 1.54 to 2.96) from the placebo geometric mean homocysteine level of 12.28μmol per L.

Author Conclusion:

- In this large-scale, placebo-controlled, randomized trial among high-risk women participants, there was no beneficial or adverse effect of a combination of 2.5mg of folic acid, 50mg of vitamin B_6, and 1mg vitamin B_{12} on a combined outcome of total major cardiovascular events in women with prior cardiovascular disease or three or more coronary risk factors over 7.3 years of follow-up. The results are consistent with prior randomized trials performed primarily among men with established vascular disease and do not support the use of folic acid and B vitamin supplements as preventive interventions for CVD in these high-risk–fortified populations.
- In subgroup analyses, there was no heterogeneity of treatment effect among those above or below the median of folate intake and among women with or without prior vascular disease. A possible interaction with randomized vitamin C and with non-randomized angiotensin-converting enzyme inhibitor treatment on the primary outcome was observed; however, due to the large number of comparisons, these results could have been due to chance.

Reviewer Comments:

- *Authors acknowledge that concerns have been raised regarding the power of the trial and others to adequately test the homocysteine hypothesis, especially in countries with folic acid fortification*
- *Authors state that although homocysteine levels were unchanged in the placebo group, folic acid fortification likely prevented further elevations in homocysteine levels that would have otherwise taken place due to the aging of the population*
- *Limitations:*
  - *The study was conducted in a population of health professionals who were at a relatively low risk of folate deficiency, and it is possible that this same regimen may have resulted in an even greater reduction in homocysteine levels in a more folate-deficient population, which might have translated into an observable benefit on cardiovascular events*
  - *The optimal dose of vitamins may be lower than the dose tested in this and other trials, and the potential for harm at higher doses has been raised by other studies*
  - *Homocysteine levels were only measured in 5% of the sample and therefore they were unable to determine whether women with high homocysteine levels at baseline may have benefited to a greater extent both with respect to homocysteine-lowering and cardiovascular events.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	No
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A

3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	Yes
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	Yes
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	Yes
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes

6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	Yes
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	Yes
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	Yes
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes

9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes